Q-221: Bacterial Community Composition In Bioaerosols From Eight California Field Sites Using An Affymetrix Microarray

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Abstract

The dynamics of airborne bacterial composition and concentration is dependent on the equilibrium of their input due to release and mixing and their removal due to stress and deposition. Sources of bacteria in the outdoor atmosphere may be local or distant. Air monitoring with pathogen detection systems is complicated by increased microbial diversity (false negatives) or the presence of close relatives (false positives). Replicated 24-h aerosol samples were collected from west to east in four areas from the coast to inland mountains across the state of California, USA. Within each broad area, a set of samples was collected from within a city and from a rural, relatively undeveloped site. Samples were amplified with universal SSU primers and the resulting products were placed on a customized Affymetrix SSU microarray. Organisms were classified by SSU sequence-specific hybridization into one of 435 bacterial sub-groups. Representatives from the Proteobacteria, the Flexibacter-Cytophaga-Bacteroides, and the gram-positive bacterial divisions as well as the Euryarchaeota division of archea were detected in both the urban and rural sites. The Fusobacteria group was only detected in the rural, coastal site where it was a major component, and Chloroflexus was only detected in the urban, mountainous site. Overall, 52 different sub-groups were detected in the urban sites while only 36 sub-groups were detected in the pristine sites. The sub-groups Streptomyces, Staphylococcus, and Clostridium limosum were found in all 8 sites while 44% of the sub-groups were found in only one or two sites. The results indicate that local reservoirs play a significant role in the bacterial community composition of bioaerosols. Increased microbial diversity of the urban sites suggests that anthropogenic sources provide additional bacterial input into the atmosphere.

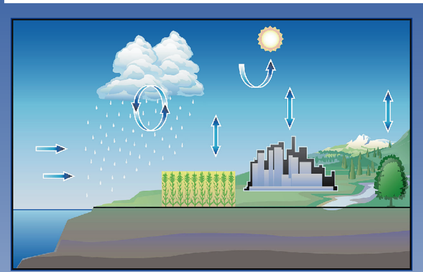


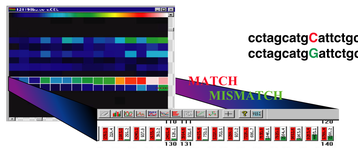
Figure 1. The microbial composition in the atmosphere is highly dynamic. Organisms are released into the air from both local and long-range sources. Meteorological and electrostatic forces influence microbial dispersal and eventual deposition. Vegetation and other localized reservoirs may influence concentration, type and distribution of organisms in outdoor environments.

Introduction

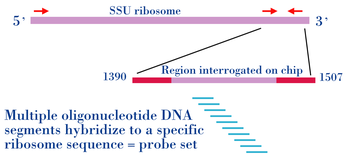
Comprehensive baseline analysis of the microbial diversity of airborne bacteria has relied exclusively on culture methods although it is estimated that greater than 99% of the organisms in air are viable but non-cultivable (VBNC). We are studying airborne microbial levels to determine if variation among natural microflora in aerosols will affect pathogen detection systems. Microbial characterization of aerosols is also important for determining the long-term effects of introducing engineered microorganisms for biopesticides and bioremediation on downwind environments. To determine the scope of microbial variation this study surveyed the diversity of organisms collected along a longitudinal transect at approximately the 39° N parallel across the state of California. Sequence variation within the 16S rRNA gene was used to provide an effective method for the identification of bacteria in environmental samples without the need for their cultivation. As an alternative to gel-based sequencing methods, we have developed a customized Affymetrix GeneChip oligonucleotide array for the identification of multiple components in a complex sample by hybridization of mixed-population DNAs to sequence-specific probes.

Approach- High Density Oligonucleotide Microarray

- Massive parallelism - 65,000 to 500,000 probes in a 1.28 cm2 array
- Identification of multiple species in a mixed population
- Organism specific region of amplified DNA products are used for targets
- Single nucleotide mismatch resolution



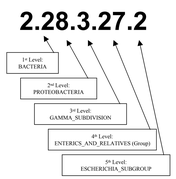
Sample reacts only with complementary signature sequences on microarray



Multiple oligonucleotide DNA segments hybridize to a specific ribosome sequence = probe set

Wilson et al. (2002) Appl. Environ. Micro. 68:2535-2541.

RDP phylocodes are used as a starting point to define probe set Operational Taxon Unit (OTU)

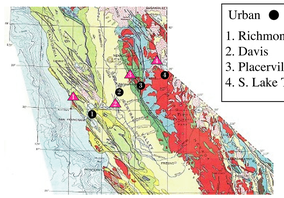


- All sequences within each terminal phylogenetic group are clustered into groups of similar sequence.
- An average of 20-30 oligonucleotide probe pairs are used for each probe set placed on a customized Affymetrix microarray.
- For an OTU to be considered present within the environmental sample, the match probe must have a significantly higher hybridization signal than the mismatch probe for a minimum of 92% of all probe pairs within the defining OTU probe set.

The microarray used in this study contains 435 OTU probe sets that identify bacterial isolates to the group level based on unique sequence within the 3' region of the 16S rRNA gene. Additional probe sets define higher level groups of eukaryotic organisms. We have recently completed a new version of this microarray that uses the full 1,500 nucleotides of the 16S rRNA gene to identify unique regions of sequence. By using 500,000 probes to identify 8,423 OTUs that differ < 2% from each other, species level identification is possible.

Location of Study

Urban/Rural Transect study

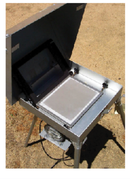


Urban	Rural
1. Richmond	1. Bodega
2. Davis	2. Jepson
3. Placerville	3. Blodgett
4. S. Lake Tahoe	4. Sagehen

An urban and rural site was selected for their location in the 1.) Coast, 2.) Central Valley, 3.) Foothills, and 4.) Mountains of California. Urban sites are all towns of less than 100,000 people; rural sites are all field stations for the University of California.

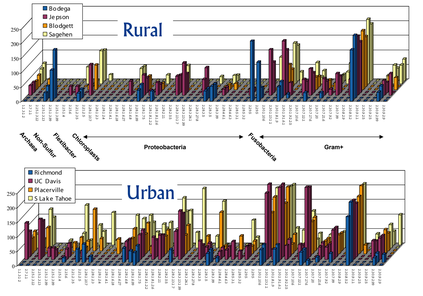
Aerosol sample collection for 24 hours

- High volume aerosol collection unit collects an average of 1361 m^3 of air (58 m^3/hr)
- Average of 1 X 10^9 bacteria/filter
- Accounts for daily bacterial fluxes that are known to occur
- Two, 24-h air samples were collected at each location in April 2002.

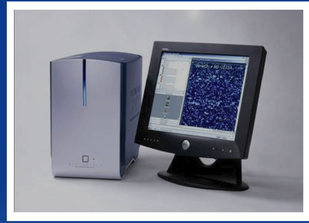


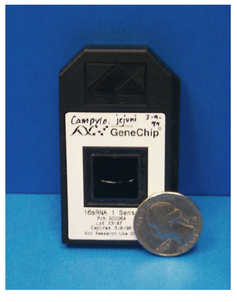
Radosevich et al. (2002) Letters in Applied Micro. 34:160-172

Bacterial Groups Detected by rDNA GeneChip



Average difference in intensity of fluorescence between probe cells (Match Probes) and control cells (Mismatch Probes) for the phylogenetic groups identified by GeneChip software. Genomic DNA was purified from filters for PCR amplification of 3' end of rRNA gene using CcompLong (TTGTACACACCGCCCGTCA, E. coli positions 1390 to 1408) and PC5B (TACCTTGTTACGACTT, E. coli positions 1507 to 1492). Two sampling dates are shown in same color for each location.





OTU of Probe Sets Detected

Group Phylogeny	Group Name	Category
1 1.1.3.1.2	Methanofollis	Archaea
2 2.7.1.1	Chloroflexus	Green Non-Sulfur
3 2.15.1.3.12	Capnocytophaga	Flexibacter
4 2.15.1.3.13	Cellulphaga	Flexibacter
5 2.15.1.3.99	Zobellia	Flexibacter
6 2.15.1.3.99	Bergeyella	Flexibacter
7 2.15.1.4	Cytophaga	Flexibacter
8 2.15.6	Cyclobacterium	Flexibacter
9 2.21.2.3.5	Algae chloroplast	Chloroplasts
10 2.21.2.5	Soybean chloroplast	Chloroplasts
11 2.28.1.10.7	Rhizomonas	α–Proteobacteria
12 2.28.1.2.3	Rhodophila	α–Proteobacteria
13 2.28.1.2.4	Gluconacetobacter	α–Proteobacteria
14 2.28.1.4.1	Rhodospirillum	α–Proteobacteria
15 2.28.1.6.18	Brucella	α–Proteobacteria
16 2.28.1.6.27	Bartonella	α–Proteobacteria
17 2.28.1.6.8	Rhodoplanes	α–Proteobacteria
18 2.28.1.6.99	Bartonella vinsonii	α–Proteobacteria
19 2.28.1.7.5	Caulobacter	α–Proteobacteria
20 2.28.1.8.1.2.2	Paracoccus	α–Proteobacteria
21 2.28.1.8.1.2.6	Rhodobacter	α–Proteobacteria
22 2.28.2.11	Duganella	β–Proteobacteria
23 2.28.2.5.5	Rhodocyclus	β–Proteobacteria
24 2.28.3.13.1.7	Acinetobacter woffii	γ–Proteobacteria
25 2.28.3.13.1.99	Acinetobacter baumannii	γ–Proteobacteria
26 2.28.3.24.1	Ruminobacter	γ–Proteobacteria
27 2.28.3.27.8	insect symbiont	γ–Proteobacteria
28 2.28.3.5	Achromatium	γ–Proteobacteria
29 2.28.3.5	Vibrio	γ–Proteobacteria
30 2.28.3.99	Pseudomonas	γ–Proteobacteria
31 2.28.4.6.1	Stigmatella	δ–Proteobacteria
32 2.28.4.6.3	Polyangium	δ–Proteobacteria
33 2.28.5.3.1	Archobacter	ε–Proteobacteria
34 2.28.5.3.2	Sulfurospirillum	ε–Proteobacteria
35 2.29.5	Fusobacterium	ε–Proteobacteria
36 2.29.6	Clostridium rectum	ε–Proteobacteria
37 2.30.1.10.8	Propionibacterium	Gram positive
38 2.30.1.13.1.1	Mycobacterium	Gram positive
39 2.30.1.8.1.9	Streptomyces	Gram positive
40 2.30.1.9.1.6.1	Renibacterium	Gram positive
41 2.30.1.9.2.2.5	Bifidobacterium	Gram positive
42 2.30.7.10.6	Bacillus badius	Gram positive
43 2.30.7.12.1	Staphylococcus	Gram positive
44 2.30.7.17.6	Lactobacillus	Gram positive
45 2.30.7.20	Enterococcus	Gram positive
46 2.30.7.21.6	Streptococcus	Gram positive
47 2.30.7.9.2	Bacillus racemilactius	Gram positive
48 2.30.7.99	Bacillus sporothermodurans	Gram positive
49 2.30.8.2.9	Clostridium innocuum	Gram positive
50 2.30.8.5.2	Mycoplasma	Gram positive
51 2.30.9.1.1	Clostridium cellobioparum	Gram positive
52 2.30.9.2.5	Clostridium botulinum	Gram positive
53 2.30.9.2.5	Clostridium thermobutyricum	Gram positive
54 2.30.9.2.99	Clostridium limosum	Gram positive
55 2.30.9.2.9	Clostridium argentinense	Gram positive

Number of prokaryotic OTUs present out of a total 435 assayed

Rural Sites

Bodega	Jepson	Blodgett	Sagehen	Total OTU
15	25	17	20	36

Urban Sites

Richmond	Davis	Placerville	S L Tahoe	Total OTU
19	31	33	39	52

Total OTUs: 55

Comparison of microbial composition in urban and rural sites

- Fusobacteria group only detected at Bodega
- Chloroflexus only detected at S. L. Tahoe
- OTUs detected at all 8 sites:
  - Streptomyces
  - Staphylococcus
  - Clostridium limosum
- 44% of OTUs (24) were found in only one or two sites

Conclusions

- Site-to-site variation suggest that local reservoirs play a significant role in bacterial community composition
- Urban sites have greater microbial diversity
- Proteobacteria and Gram (+) are predominant component of bioaerosols
- Sequence specific hybridization of ribosomal DNA/RNA to microarray allows detailed information on microbial composition and diversity for any environmental sample

Acknowledgements

We thank K. H. Wilson and J. H. Shinn for assistance in experimental design. We gratefully acknowledge the valuable technical assistance of T. A. Kuczmarski, J. L. Radosevich and K. M Stivers. This work was funded by the Chemical and Biological Non-Proliferation program, NN-22 for the Department of Energy.